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attachment to
Paper # 15

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  *                W E L C O M E   T O   T H E                    *
  *          U. S.   P A T E N T   T E X T   F I L E              *
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=> glutamine synthetase?

'GLUTAMINE' IS NOT A RECOGNIZED COMMAND

=> s glutamine synthetase?

          8424 GLUTAMINE
          2453 SYNTHETASE?
L1         173 GLUTAMINE SYNTHETASE?
                 (GLUTAMINE(W)SYNTHETASE?)

=> s l1 and hamster

          3540 HAMSTER
L2          52 L1 AND HAMSTER

=> s l2 and vector?

         60690 VECTOR?
L3          49 L2 AND VECTOR?

=> d l3,1-49,cit
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1. 5,679,546, Oct. 21, 1997, Chimeric proteins which block complement activation; Jone-Long Ko, et al., 435/69.2, 69.7, 252.3, 320.1; 530/350, 412; 536/23.4 [IMAGE AVAILABLE]

2. 5,676,943, Oct. 14, 1997, Compositions and methods for the delivery of biologically active molecules using genetically altered cells contained in biocompatible immunoisolatory capsules; Edward E. Baetge, et al., 424/93.21, 93.3; 435/172.3 [IMAGE AVAILABLE]

3. 5,674,834, Oct. 7, 1997, Stable bactericidal/permeability-increasing protein products and pharmaceutical compositions containing the same; Georgia Theofan, et al., 514/2, 21; 530/350 [IMAGE AVAILABLE]

4. 5,670,623, Sep. 23, 1997, Methods of use of cellulose binding domain proteins; Oded Shoseyov, et al., 530/350; 436/530, 828; 530/390.1, 390.5, 415, 417, 820, 861 [IMAGE AVAILABLE]

5. 5,658,763, Aug. 19, 1997, Methods and compositions for high protein production from non-native DNA; Haimanti Dorai, et al., 435/69.7; 530/387.1, 387.3; 536/23.53 [IMAGE AVAILABLE]

6. 5,656,481, Aug. 12, 1997, Compositions and methods for the delivery of biologically active molecules using cells contained in biocompatible capsules; Edward E. Baetge, et al., 435/325; 424/93.1, 93.2, 93.21, 93.3, 93.7; 435/172.3, 347, 373, 382 [IMAGE AVAILABLE]

7. 5,653,975, Aug. 5, 1997, Compositions and methods for the delivery of

biologically active molecules using cells contained in biocompatible capsules; Edward E. B[redacted]ge, et al., 424/93.1, 93.2, 93[redacted], 93.3, 93.7; 435/172.3 [IMAGE AVAILABLE]

8. 5,652,138, Jul. 29, 1997, Human neutralizing monoclonal antibodies to human immunodeficiency virus; Dennis R. Burton, et al., 435/252.33; 424/142.1, 148.1, 160.1; 435/69.6, 172.3, 320.1; 530/388.15, 388.35, 389.4 [IMAGE AVAILABLE]

9. 5,644,036, Jul. 1, 1997, Purified immunoglobulin; Paul Ian Nicholas Ramage, et al., 530/412; 435/69.6; 530/413, 416, 417 [IMAGE AVAILABLE]

10. 5,639,275, Jun. 17, 1997, Delivery of biologically active molecules using cells contained in biocompatible immunoisolatory capsules; Edward E. Baetge, et al., 604/891.1; 424/93.1, 93.2, 422, 424; 435/172.3 [IMAGE AVAILABLE]

11. 5,631,158, May 20, 1997, Methods and compositions for high protein production from non-native DNA; Haimanti Dorai, et al., 435/172.3, 70.21, 172.2, 252.3, 320.1; 530/387.3, 867; 536/23.53, 23.72, 24.1 [IMAGE AVAILABLE]

12. 5,627,047, May 6, 1997, Astrocyte-specific transcription of human genes; Michael Brenner, et al., 435/69.1, 69.7, 320.1, 325, 354, 368; 536/23.4, 23.5, 24.1 [IMAGE AVAILABLE]

13. 5,627,033, May 6, 1997, Mammalian expression **vectors**; John M. Smith, et al., 435/6, 91.41, 172.3, 320.1, 325, 358, 365 [IMAGE AVAILABLE]

14. 5,623,053, Apr. 22, 1997, Soluble mammal-derived Fc receptor which binds at a pH ranging from about 5.5 to 6.5 and releases at a pH ranging from about 7.5 to 8.5; Louis N. Gastinel, et al., 530/350; 435/69.1 [IMAGE AVAILABLE]

15. 5,614,385, Mar. 25, 1997, Methods and compositions for high protein production from recombinant DNA; Hermann Oppermann, et al., 435/69.4, 254.2, 325 [IMAGE AVAILABLE]

16. 5,612,213, Mar. 18, 1997, Method of selecting mammalian cell lines having improved productivity; Sham Y. Chan, 435/6, 69.1, 320.1, 325, 366, 369 [IMAGE AVAILABLE]

17. 5,605,690, Feb. 25, 1997, Methods of lowering active TNF-.alpha. levels in mammals using tumor necrosis factor receptor; Cindy A. Jacobs, et al., 424/134.1; 435/69.7; 514/12, 825; 530/350, 387.3, 866, 868 [IMAGE AVAILABLE]

18. 5,599,984, Feb. 4, 1997, Guanylhydrazones and their use to treat inflammatory conditions; Marina Bianchi, et al., 564/157; 546/332; 564/48, 50, 51, 148, 151, 155, 227, 228, 236 [IMAGE AVAILABLE]

19. 5,599,788, Feb. 4, 1997, Method for accelerating skin wound healing with H3 protein; Anthony F. Purchio, et al., 514/2; 424/278.1, 409; 514/12, 885, 886, 887, 944, 945, 946, 947 [IMAGE AVAILABLE]

20. 5,591,630, Jan. 7, 1997, Monoclonal antibodies that bind interleukin-15 receptors; Dirk M. Anderson, et al., 435/331, 334; 530/388.22 [IMAGE AVAILABLE]

21. 5,589,374, Dec. 31, 1996, Diabetogene rad: a type II diabetes specific gene; C. Ronald Kahn, et al., 435/69.1, 252.3, 320.1; 536/23.2, 23.5 [IMAGE AVAILABLE]

22. 5,585,237, Dec. 17, 1996, Methods and compositions for high protein

production from recombinant DNA; Hermann Oppermann, et al., 435/6, 172.3, 325, 350, 353, 358 [IMAGE AVAILABLE]

23. 5,580,723, Dec. 3, 1996, Method for identifying active domains and amino acid residues in polypeptides and hormone variants; James A. Wells, et al., 435/6, 7.1, 69.1, 71.1; 436/501; 530/387.1, 388.1, 399, 806, 808; 935/10, 11, 12, 13, 14, 15, 76, 77, 82 [IMAGE AVAILABLE]

24. 5,578,461, Nov. 26, 1996, Gene manipulation and expression using genomic elements; Stephen Sherwin, et al., 435/69.1, 172.3, 244, 320.1; 536/23.1, 24.1; 935/28, 33, 55 [IMAGE AVAILABLE]

25. 5,561,053, Oct. 1, 1996, Method for selecting high-expressing host cells; Craig W. Crowley, 435/69.1, 172.3, 320.1, 358; 536/23.2 [IMAGE AVAILABLE]

26. 5,545,723, Aug. 13, 1996, Muteins of IFN-.beta.; Susan E. Goelz, et al., 424/85.6; 435/69.51, 252.3, 320.1; 514/12; 530/351; 536/23.52 [IMAGE AVAILABLE]

27. 5,545,405, Aug. 13, 1996, Method for treating a mammal suffering from cancer with a cho-glycosylated antibody; Martin J. Page, 424/133.1, 130.1, 143.1, 172.1, 174.1; 435/70.3, 71.1, 320.1; 530/387.1, 388.1, 388.22, 388.73, 388.75, 389.1, 389.6, 389.7 [IMAGE AVAILABLE]

28. 5,545,404, Aug. 13, 1996, Method for treating a mammal suffering from a T-cell medicated disorder with a CHO-Glycosylated antibody; Martin J. Page, 424/133.1, 130.1, 143.1, 173.1, 174.1; 435/70.3, 71.1, 320.1; 530/387.1, 388.22, 388.73, 388.75, 388.8, 389.1, 389.6, 389.7 [IMAGE AVAILABLE]

29. 5,545,403, Aug. 13, 1996, Method for treating a mammal by administering a CHO-glycosylated antibody; Martin J. Page, 424/133.1, 130.1, 135.1, 136.1, 138.1, 143.1, 147.1, 150.1, 159.1, 172.1, 174.1; 435/70.3, 71.1, 320.1; 530/387.1, 388.1, 388.22, 388.73, 388.75, 389.1, 389.6, 389.7 [IMAGE AVAILABLE]

30. 5,496,934, Mar. 5, 1996, Nucleic acids encoding a cellulose binding domain; Oded Shoseyov, et al., 536/23.7; 435/252.3, 320.1; 536/23.1, 24.33 [IMAGE AVAILABLE]

31. 5,468,845, Nov. 21, 1995, Antibodies to osteogenic proteins; Hermann Oppermann, et al., 530/387.9, 350 [IMAGE AVAILABLE]

32. 5,464,937, Nov. 7, 1995, Type II Interleukin-1 receptors; John E. Sims, et al., 530/350 [IMAGE AVAILABLE]

33. 5,447,913, Sep. 5, 1995, Therapeutic uses of bactericidal/permeability-increasing protein dimer products; William S. Ammons, et al., 514/12, 21; 530/350 [IMAGE AVAILABLE]

34. 5,427,940, Jun. 27, 1995, Engineered cells producing insulin in response to glucose; Christopher B. Newgard, 435/366; 424/520; 435/4, 6, 69.1, 172.1, 172.2, 172.3, 320.1; 530/303, 350, 389.2, 397 [IMAGE AVAILABLE]

35. 5,420,247, May 30, 1995, Leukemia inhibitory factor receptors and fusion proteins; David P. Gearing, et al., 530/350, 387.3, 388.23, 391.1, 402; 536/23.51 [IMAGE AVAILABLE]

36. 5,420,019, May 30, 1995, Stable bactericidal/permeability-increasing protein muteins; Georgia Theofan, et al., 435/69.1, 252.3, 320.1; 530/350; 536/23.5 [IMAGE AVAILABLE]

37. 5,395,760, Mar. 7, 1995, DNA encoding tumor necrosis factor-.alpha.

and -.beta. receptors; Craig A. Smith, et al., 435/365; 424/85.1; 435/69.4, 172.3; 530/[redacted], 388.23; 536/23.51 [IMAGE AVA[redacted]BLE]

38. 5,376,567, Dec. 27, 1994, Expression of interferon genes in Chinese **hamster** ovary cells; Francis P. McCormick, et al., 435/320.1; 424/85.4; 435/69.51, 91.41, 252.3, 358; 536/23.52; 935/23, 56 [IMAGE AVAILABLE]

39. 5,354,557, Oct. 11, 1994, Osteogenic devices; Hermann Oppermann, et al., 424/423, 422, 424, 426 [IMAGE AVAILABLE]

40. 5,350,683, Sep. 27, 1994, DNA encoding type II interleukin-1 receptors; John E. Sims, et al., 435/69.1, 252.3, 320.1; 530/350; 536/23.5 [IMAGE AVAILABLE]

41. 5,284,755, Feb. 8, 1994, DNA encoding leukemia inhibitory factor receptors; David P. Gearing, et al., 435/69.1, 69.7, 252.3, 320.1; 536/23.4, 23.5 [IMAGE AVAILABLE]

42. 5,266,683, Nov. 30, 1993, Osteogenic proteins; Hermann Oppermann, et al., 530/326, 327, 328, 350, 395, 840 [IMAGE AVAILABLE]

43. 5,122,464, Jun. 16, 1992, Method for dominant selection in eucaryotic cells; Richard H. Wilson, et al., 435/172.3, 320.1 [IMAGE AVAILABLE]

44. 5,098,703, Mar. 24, 1992, Interferon-alpha 76; Michael A. Innis, 424/85.7; 435/69.51, 811; 530/351; 536/23.52 [IMAGE AVAILABLE]

45. 5,043,270, Aug. 27, 1991, Intronic overexpression **vectors**; John M. Abrams, et al., 435/69.1, 172.3, 320.1, 358; 536/23.2, 23.5; 935/34, 61, 66, 70, 71, 79, 84 [IMAGE AVAILABLE]

46. 4,975,276, Dec. 4, 1990, Interferon-alpha 54; Michael A. Innis, 424/85.7, 85.4; 435/69.51, 811; 530/351 [IMAGE AVAILABLE]

47. 4,973,479, Nov. 27, 1990, Interferon-.alpha.61; Michael A. Innis, 424/85.7, 85.4; 435/69.51, 811; 530/351 [IMAGE AVAILABLE]

48. 4,966,843, Oct. 30, 1990, Expression of interferon genes in Chinese **hamster** ovary cells; Francis P. McCormick, et al., 435/69.51, 70.1, 70.3, 70.5, 172.1, 172.3, 320.1, 360, 811; 536/23.5, 23.52, 24.1; 935/11, 34, 70 [IMAGE AVAILABLE]

49. 4,956,288, Sep. 11, 1990, Method for producing cells containing stably integrated foreign DNA at a high copy number, the cells produced by this method, and the use of these cells to produce the polypeptides coded for by the foreign DNA; James G. Barsoum, 435/172.3, 69.1, 70.1, 71.1, 172.1, 252.3; 935/16, 33, 52 [IMAGE AVAILABLE]

=> d 16,clms

US PAT NO: 5,612,213 [IMAGE AVAILABLE] L3: 16 of 49

CLAIMS:

CLMS(1)

What is claimed is:

1. A process of selecting a cell line having high heterologous protein productivity from a population of cells at least some of which may include an endogenous sequence for an amplifiable marker and some of which do not include the endogenous sequence for the same marker,

comprising:
A. co-transfecting t[redacted]population of mammalian cells [redacted]h DNA sequences coding for the heterologous protein and an amplifiable marker, and a DNA sequence encoding a selectable marker;
B. further cloning the transfected cells into subpopulations in a medium containing a selective agent and selecting for such selectable marker resistant cells;
C. assaying the selectable marker resistant cells for heterologous protein productivity;
D. culturing said selectable marker resistant cells in media containing an amplifying agent, wherein the host cell includes the sequence for the amplifiable marker;
E. establishing the amplifiability index AE for each cell population, said index being defined by the equation

.DELTA.E=Y/X

wherein X is the productivity of cells before a first amplification, and Y is the productivity of cells after said first amplification;
F. selecting and further amplifying cells with a .DELTA.E index value of greater than 3, said amplifying step being performed in increasing amounts of amplifying agent; and
G. selecting at least one cell line having high productivity from the cells of step F.

CLMS(2)

2. The method of claim 1 wherein said host cells comprise an expression **vector** having a sequence of double stranded DNA including:
A. an augmenting sequence downstream of a promoter and upstream of a DNA encoding the amino acid sequence of the desired heterologous protein;
B. DNA encoding the amino acid sequence of the desired protein downstream of said augmenting sequence; and
C. DNA coding a polyadenylation sequence downstream of a transcription termination site.

CLMS(3)

3. The method of claim 2 wherein the augmenting sequence comprises in linear sequence a splice donor.sub.1 -intron.sub.1 -splice donor.sub.2 -intron.sub.2 -acceptor sequence operatively linked to the desired heterologous protein.

CLMS(4)

4. The method of claim 2 wherein the promoter is from the immediate early gene of human cytomegalovirus.

CLMS(5)

5. The method of claim 3 wherein the splice donor.sub.1 -intron.sub.1 sequence is from the major late promoter region of adenovirus.

CLMS(6)

6. The method of claim 3 wherein the splice donor sequence is from the human cytomegalovirus.

CLMS(7)

7. The method of claim 3 wherein the intron.sub.2 -acceptor sequence is from the human immunoglobulin variable region.

CLMS(8)

8. The method of claim 1 wherein the DNA sequence coding for the

heterologous protein encodes Factor VIII.

CLMS(9)

9. The method of claim 1 wherein the host cell is a human cell.

CLMS(10)

10. The method of claim 1 wherein the host cell is a human embryonic kidney cell.

CLMS(11)

11. The method of claim 1 wherein the host cell is a human embryonic kidney 293s cell.

CLMS(12)

12. A **vector** comprising in linear sequence a second promoter-first promoter-splice donor.sub.1 -intron.sub.1 -splice donor.sub.2 -intron.sub.2 -acceptor sequence operatively linked to a protein coding sequence.

CLMS(13)

13. The **vector** pCAIS-F8 chromosomally integrated into a mammalian cell.

CLMS(14)

14. The **vector** of claim 12 comprising a coding sequence for Factor VIII located downstream from the acceptor.

CLMS(15)

15. A mammalian cell line comprising the **vector** of claim 12 as an integrated chromosomal element.

CLMS(16)

16. A mammalian cell line comprising the **vector** of claim 14 as an integrated chromosomal element.

```
=> s l3 and medium(5A)lack?(5A)glutamine

        356292 MEDIUM
        154162 LACK?
          8424 GLUTAMINE
            11 MEDIUM(5A)LACK?(5A)GLUTAMINE
L4           7 L3 AND MEDIUM(5A)LACK?(5A)GLUTAMINE

=> d l4,1-7,cit,ab
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1. 5,674,834, Oct. 7, 1997, Stable bactericidal/permeability-increasing protein products and pharmaceutical compositions containing the same; Georgia Theofan, et al., 514/2, 21; 530/350 [IMAGE AVAILABLE]

US PAT NO: 5,674,834 [IMAGE AVAILABLE] L4: 1 of 7

ABSTRACT:
Disclosed are novel bactericidal/permeability-increasing (BPI) protein products wherein cysteine residue number 132 or 135 is replaced by another amino acid residue, preferably an alanine or serine residue and/or wherein the leucine residue at position 193 is the carboxy

terminal residue. Also disclosed are DNA sequences encoding methods for the production of the [redacted]me in appropriate host cells, [redacted] stable homogeneous pharmaceutical compositions containing the analogs suitable for use treatment of gram negative bacterial infection and its sequelae.

2. 5,545,405, Aug. 13, 1996, Method for treating a mammal suffering from cancer with a cho-glycosylated antibody; Martin J. Page, 424/133.1, 130.1, 143.1, 172.1, 174.1; 435/70.3, 71.1, 320.1; 530/387.1, 388.1, 388.22, 388.73, 388.75, 389.1, 389.6, 389.7 [IMAGE AVAILABLE]

US PAT NO: 5,545,405 [IMAGE AVAILABLE] L4: 2 of 7

ABSTRACT:
The invention relates to a CHO cell-line capable of producing antibody, the cell-line having been co-transfected with a **vector** capable of expressing the light chain of the antibody and a **vector** capable of expressing the heavy chain of the antibody wherein the **vectors** contain independently selectable markers; also included is a CHO cell-line capable of producing a human antibody or an altered antibody, the cell-line having been transfected with a **vector** capable of expressing the light chain of the antibody and the heavy chain of the antibody; process for the production of antibody using a CHO cell-line and antibody having CHO glycosylation.

3. 5,545,404, Aug. 13, 1996, Method for treating a mammal suffering from a T-cell medicated disorder with a CHO-Glycosylated antibody; Martin J. Page, 424/133.1, 130.1, 143.1, 173.1, 174.1; 435/70.3, 71.1, 320.1; 530/387.1, 388.22, 388.73, 388.75, 388.8, 389.1, 389.6, 389.7 [IMAGE AVAILABLE]

US PAT NO: 5,545,404 [IMAGE AVAILABLE] L4: 3 of 7

ABSTRACT:
The invention relates to a CHO cell-line capable of producing antibody, the cell-line having been co-transfected with a **vector** capable of expressing the light chain of the antibody and a **vector** capable of expressing the heavy chain of the antibody wherein the **vectors** contain independently selectable markers; also included is a CHO cell-line capable of producing a human antibody or an altered antibody, the cell-line having been transfected with a **vector** capable of expressing the light chain of the antibody and the heavy chain of the antibody; process for the production of antibody using a CHO cell-line and antibody having CHO glycosylation.

4. 5,545,403, Aug. 13, 1996, Method for treating a mammal by administering a CHO-glycosylated antibody; Martin J. Page, 424/133.1, 130.1, 135.1, 136.1, 138.1, 143.1, 147.1, 150.1, 159.1, 172.1, 174.1; 435/70.3, 71.1, 320.1; 530/387.1, 388.1, 388.22, 388.73, 388.75, 389.1, 389.6, 389.7 [IMAGE AVAILABLE]

US PAT NO: 5,545,403 [IMAGE AVAILABLE] L4: 4 of 7

ABSTRACT:
The invention relates to a CHO cell-line capable of producing antibody, the cell-line having been co-transfected with a **vector** capable of expressing the light chain of the antibody and a **vector** capable of expressing the heavy chain of the antibody wherein the **vectors** contain independently selectable markers; also included is a CHO cell-line capable of producing a human antibody or an altered antibody, the cell-line having been transfected with a **vector** capable of expressing the light chain of the antibody and the heavy chain of the antibody; process for the production of antibody using a CHO cell-line and antibody having CHO glycosylation.

5. 5,420,019, May 30, 1995, Stable bactericidal/permeability-increasing

protein muteins; Georgia Theofan, et al., 435/69.1, 252.3, 320.1; 530/350; 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,420,019 [IMAGE AVAILABLE] L4: 5 of 7

ABSTRACT:
Disclosed are novel bactericidal/permeability-increasing (BPI) protein products wherein cysteine residue number 132 or 135 is replaced by another amino acid residue, preferably an alanine or serine residue and/or wherein the leucine residue at position 193 is the carboxy terminal residue. Also disclosed are DNA sequences encoding methods for the production of the same in appropriate host cells, and stable homogeneous pharmaceutical compositions containing the analogs suitable for use treatment of gram negative bacterial infection and its sequelae.

6. 5,122,464, Jun. 16, 1992, Method for dominant selection in eucaryotic cells; Richard H. Wilson, et al., 435/172.3, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,122,464 [IMAGE AVAILABLE] L4: 6 of 7

ABSTRACT:

Recombinant DNA sequences which encode the complete amino acid sequence of a **glutamine synthetase, vectors** containing such sequences, and methods for their use, in particular as dominant selectable markers, for use in co-amplificiation of non-selected genes and in transforming host cell lines to glutamine independence.

7. 4,956,288, Sep. 11, 1990, Method for producing cells containing stably integrated foreign DNA at a high copy number, the cells produced by this method, and the use of these cells to produce the polypeptides coded for by the foreign DNA; James G. Barsoum, 435/172.3, 69.1, 70.1, 71.1, 172.1, 252.3; 935/16, 33, 52 [IMAGE AVAILABLE]

US PAT NO: 4,956,288 [IMAGE AVAILABLE] L4: 7 of 7

ABSTRACT:
An improved method, employing electroporation, for producing novel recombinant host cells characterized by stably integrated foreign DNA at high copy number. These recombinant host cells are useful in the efficient, large-scale production of recombinant proteins and polypeptides.